Technews

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Antimicrobials Residues in Milk from Animal Origin

This bulletin includes technical information based on latest developments on products, systems, techniques etc. reported in journals, companies' leaflets and books and based on studies and experience. The technical information in different issues is on different areas of plant operation. It is hoped that the information contained herein will be useful to readers.

The theme of information in this issue "**Antimicrobials residues in milk from animal origin"**. It may be understood that the information given here is by no means complete.

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INTRODUCTION

Antimicrobials are compounds exhibit selective antimicrobial activity. **Veterinary drugs** are pharmacologically and biologically active chemical agents especially designed for the treatment and prevention of animal diseases. Some are active against many gram-positive bacteria, others predominantly against gram negatives, and a few, the broad-spectrums, are inhibitors of members of both groups. Some others are antifungal only.

Some compounds, such as aminoglycosides, bacteriocins and penicillin, are isolated from living organisms, whereas others, such as oxazolidinones, quinolones, and sulfonamides, are produced by chemical synthesis. Accordingly, antibiotics can be classified based on their as natural, semisynthetic, or synthetic. Most of the common antibiotics used today are semisynthetic modifications of a variety of natural compounds. The use of antibiotics in the treatment and prevention of bacterial disease in livestock production follows similar principles to those used in the practice of human medicine, but with subtle difference.

Antibiotics get administered to animals by injection or orally via feed and water to livestock and poultry. Antibiotics not only allow the growth of healthier and more productive farm animals through improved weight gain and feed conversion efficiency, but they are also effective against animal diseases. The prolonged use at low concentration encourages the production of antibiotic resistant strains of bacteria.

Other non-antibiotic antimicrobials are also used in foodborne pathogen prevention. These strategies include vaccination and the use of bacteriocins, bacteriophages, enzymes, probiotics, prebiotics, and organic acids. Although the employment of antimicrobial agents has multiple significant benefits in animal and agriculture, the appropriate use of these agents, including;

a. how to select the right ones
b. how to administrate them and
c. how to assess their risks

Is a highly complex issue and continues to be a challenge for most growers and farmers.

It is widely known that improper use of antibiotics may lead to residues in milk, especially when withdrawal times are not respected. These residues can be dangerous for human health. They may cause allergic reactions, antibiotic resistance of pathogens etc. **Antibiotic residues** can also create a technological problem for industry production concerning bacterial fermentation processes in dairy products.

Nowadays there are several receptor-based lateral flow assay tests employed routinely at the farm level and in the dairy industry because they are fast and simple to use. Microbiological test kits based on the microbial inhibition are most frequently used for the screening analysis of milk in farms and dairy industries.

USE OF ANTIBIOTICS IN DAIRY FARMING

Antibiotics used as veterinary medicine

The therapeutic treatment of individual sick animals with antibiotics or other effective antimicrobials is essential and is employed all over the world. There are three major therapeutic patterns of antibiotic use in livestock:

1. Prophylaxis- which targets exposed healthy animals before onset of risk diseases

2. Metaphylaxis- which is the mass treatment of animal populations currently suffering from diseases before the onset of blatant illness and

3. Treatment for animals experiencing acute clinical diseases.

The dose regimen for these three therapeutic uses of antibiotics relies on the expected minimum inhibitory concentration of the target pathogens expected to be implicated.

Antibiotics used for veterinary therapy are often administered orally through feed and water, or by injection, in order to relieve animals suffering and reduce production losses. Broad-spectrum or combinations of antibiotics are commonly used in such situations when the specific pathogens of concern are unidentified or in doubt. However, a narrow-spectrum antibiotic able to target a specific pathogen involved in animal disease should be the

first choice and could also lower the risk level of antibiotic resistance.

FDA approved drugs for lactating cows is given in table.

Active ingredients	Milk withholding time	Product name	Manufacturer/Marketer
Injectable Use			
Ampicillin trihydrate	48hrs	Polyflex®	Boehringer Ingelheim Vetmedica, Inc.
Ceftiofur crystalline-free acid	None	EXCEDE®	Zoetis, Inc.
Ceftiofur hydrochloride	None	EXCENEL® RTU	Zoetis, Inc.
Ceftiofur sodium	None	Naxcel® Sterile Powder	Zoetis, Inc.
Cloprostenol sodium	None	Estrumate	Merck Animal Health
Dexamethasone	None	Dexamethasone Solution	Phoenix Pharmaceutical, Inc./Clipper Distributing
	None	Dexium	Bimeda, Inc.
Dinoprost tromethamine	None	Lutalyse® Sterile Solution	Zoetis, Inc.
	None	ProstaMate®	Bayer HealthCare LLC, Animal Health
Flunixin meglumine	36hrs	Flu-Nix D Injection	Agri Laboratories, Ltd.
	36hrs	Banamine	Merck Animal Health
	36hrs	Flunazine	Bimeda, Inc.
	36hrs	Flunixin Injection	Norbrook Laboratories, Ltd.
Gonadorelin diacetate tetrahydrate	None	Cystorelin Injectable	Merial Limited
	None	Fertagyl®	Merck Animal Health
	None	OvaCyst®	Bayer HealthCare LLC, Animal Health
Gonadorelin hydrochloride	None	Factrel®	Zoetis, Inc.
Gonadotropin (chorionic)	None	Chorulon®	Merck Animal Health for Chorulon (CG)
Isoflupredone acetate	None	Predef® 2x	Zoetis, Inc.
Oxytetracycline	96hrs	Agrimycin 200	Agri Laboratories, Ltd.
	96hrs	Bio-Mycin® 200	Boehringer Ingelheim Vetmedica, Inc.
	96hrs	Oxytetracycline Injection 200	Norbrook Laboratories, Ltd.
	96hrs	Pennox 200 Injectable	Pennfield Animal Health
	96hrs	Liquamycin® LA-200®	Zoetis, Inc.
Oxytocin	None	Oxytocin Injection	Bimeda, Inc.

Penicillin G (procaine)	48hrs	Agri-Cillin Injection	Agri Laboratories, Ltd.
	48hrs	Pro-Pen-G™ Injection	Bimeda, Inc
	48hrs	Hanford's/US Vet Sterile Penicillin G Penicillin G Procaine aqueous Suspension	Norbrook Laboratories, Ltd.
	48hrs	Norocillin	Norbrook Laboratories, Ltd.
Sometribove zinc	None	Posilac	Elanco Animal Health
Sulfadimethoxine	60hrs	Di-Methox Injection 40%	Agri Laboratories, Ltd.
Tripelennamine hydrochloride	24hrs	Recovr Injectable	Zoetis, Inc.
Intra Mammary Use			
Amoxicillin trihydrate	60 hrs	Amoxi-Mast®	Merck Animal Health
Ceftiofur hydrochloride	72 hrs	SPECTRAMAST™ LC	Zoetis, Inc.
Cephapirin (sodium)	96 hrs	Today®	Boehringer Ingelheim Vetmedica, Inc.
Cloxacillin (sodium)	48 hrs	Dariclox®	Merck Animal Health
Hetacillin (potassium)	72 hrs	Hetacin®K;	Boehringer Ingelheim Vetmedica, Inc.
Penicillin G (procaine)	60 hrs	Hanford's/US Vet MASTICLEAR™	G.C. Hanford Mfg. Co.
Pirlimycin	36 hrs	Pirsue® Sterile Solution	Zoetis, Inc.
Oral Use			
Fenbendazole	72 hrs	Safe-Guard 10% Paste	Merck Animal Health
	None	Safe-Guard 10%-Suspension	Merck Animal Health
Magnesium hydroxide	12hrs	Carmilax Bolus	Zoetis, Inc.
	12hrs	Carmilax Powder	
Poloxalene	None	Bloat Guard® Top Dressing	Phibro Animal Health
		TheraBloat® Drench Concentrate	Zoetis, Inc.
Sulfadimethoxine	60hrs	ALBON® Bolus	Zoetis, Inc.
Feed Additive Use			
Fenbendazole	None	Safe-Guard 0.5% Top Dress Pellets	Merck Animal Health
	None	Safe-Guard 1.96%	Merck Animal Health
	None	Safe-Guard 20% Salt Free-Choice Mineral	Merck Animal Health
	None	Safe-Guard 35% Salt Free-Choice Mineral	Merck Animal Health
Monensin (sodium)	None	Rumensin 90	Elanco Animal Health
Morantel tartrate	None	Rumatel® 88	Phibro Animal Health

Poloxalene	None	Bloat Guard® Liquid T ype A Medicated Article	Phibro Animal Health
	None	Bloat Guard® Medicated top dressing	Phibro Animal Health
	None	Bloat Guard® Type A Medicated Article	Phibro Animal Health
Intra-vaginal Administration			
Progesterone	None	EAZI-Breed™ CIDR® Cattle Insert	Zoetis, Inc.
Topical Use			
Balsam peru oil	None	Granulex Liquid	UDL Laboratories, Inc.
Castor oil	None	Granulex Liquid	UDL Laboratories, Inc.
Eprinomectin	None	Ivomec® Eprinex® Pour-On for Beef & Dairy Cattle	Merial Limited
Moxidectin	None	Cydectin® (moxidectin) 0.5% Pour-On for Cattle	Boehringer Ingelheim Vetmedica, Inc.
Oxytetracycline hydrochloride/Polymyxin B sulfate	None	Terramycin® Ophthalmic Ointment with Polymyxin	Zoetis, Inc.
Trypsin	None	Granulex Liquid	UDL Laboratories, Inc.

Antibiotics used as growth promoters

Generally, 'growth promoter' refers to products that help to grow an animal faster for the same unit amount of feed consumed in a given period of time. Several researchers have shown that low-concentration (usually 2.5–50 mg/ kg) addition of antibiotics to animal feed results in an accelerated growth rate and improved feed conversion efficiency in agricultural animals such as cattle, pigs, sheep, and poultry. The studies show that there may be up to 10% gain in both weight and feed conversion efficiency. The prolonged use at low concentration encourages the production of antibiotic resistant strains of bacteria.
One example is the emergence of fluoroquinolone resistant *Campylobacter*, one of several bacterial species that cause severe food poisoning in humans.

CAUSES OF ANTIBIOTIC RESIDUES IN MILK & MEAT

Drug residues can be avoided by a well-planned drug use program. Reasons given for antibiotic residues in milk is the result of many on-farm situations. These include, but are not limited to, the following:

1. Lack of consultation from a licensed veterinarian.
2. Not following veterinarian's recommendation when using any drug.
3. Not following the manufacturer or veterinarian–prescribed label directions for correct treatment.
4. Not following the manufacturer or veterinarian–prescribed label directions for the appropriate withdrawal period.
5. Poor identification of all cattle including bull calves.
6. Accidentally milking a treated cow into the bulk tank or not diverting from bulk tank.
7. Long-term residue following treatment as a calf.
8. Use of medicated milk replacers in calves.

SOURCE OF ANTIBIOTICS IN MILK

Antibiotics employed for infectious disease prevention and treatment in large groups of farm animals such as cattle, swine, and chicken are usually administered orally in drinking water or as feed additives, and sometimes also via intra-mammary infusions. A frequent and prevailing source of the milk contamination is the intra-mammary administration of a specific antibiotic. Other pathways for

the milk contamination are cutaneous, intrauterine, subcutaneous, intramuscular, and intravenous drug administrations.

1. Mastitis is a major problem in dairy cattle and can impair normal lactation. Pathogens including *Pseudomonas*, *Staphylococcus*, *Mycoplasma*, *Pasteurella*, *E. coli,* and *Streptococcus* cause mastitis. Mastitis is treated with antibiotics delivered directly into the udder and sometimes injecting those by parenteral routes. Treated cows are required to be excluded from the milk supply for a specific time period to ensure that antibiotic residues are not excreted in their milk.

2. The health of cow and udder also has profound effect on excretion of antibiotics in milk. The fibrosis of udder tissue in chronic mastitis leading to poor distribution and absorption of penicillin cause higher concentrations and longer retention of penicillin in milk of the affected quarters compared to healthy quarters.

3. Improper management and cleaning practices of equipment's during milking of treated cows.

4. Intravenous infusion of high dosages of ceftiofur in cows with experimentally induced *Escherichia coli* mastitis resulted in significantly longer excretion of ceftiofur in milk compared to healthy cows.

5. Feed concentrates containing sulfadiazine, sulfadimidine or chlortetracycline at levels (250 mg/kg) normally used for treatment to dairy cattle for 21 days. Carryover of sulfonamides in milk were in the range around 100µg/l.

6. Three low levels of nicarbazin, meticlorpindol and ivermectin, respectively, to both high and low-producing dairy cattle for 21 days.

    a. Nicarbazin could not be detected in milk.

    b. Meticlorpindol was found in milk in concentrations between 5 and 50µg/kg during the feeding period and less than 5µg/kg immediately post-exposure.

    c. Up to 7 µg/kg Concentrations of ivermectin was found in milk throughout the whole exposure period and 10 days of post exposure.

7. Oral administration of Oxfendazole and albendazole to dairy cattles, Oxfendazole and oxidized and hydroxylated metabolite concentrations in milk were less than 1 µg/ml. The parent albendazole was not found in milk and the metabolite (sulfoxide and sulfone) concentrations were less than 1 µg/ml.

Maximum Residue Limits (MRL) for commonly used veterinary drugs in cow milk (Codex Alimentarius Commission 2012)		
Name of Antibiotics	**Class of Drugs**	**MRL(µg/l)**
Albendazole	Benzimidazole anthelmintic	100
Amoxicillin	Penicillin antibiotic	4
Benzylpenicillin/Procaine Benzylpenicillin	Penicillin antibiotic	4
Ceftiofur	Cephalosporin antibiotic	100
Chlortetracycline/ Oxytetracycline /Tetracycline	Tetracycline antibiotic	100
Colistin	Polymyxin antibiotic	50
Dihydrostreptomycin /Streptomycin	Aminoglycoside antibiotic	200
Diminazene	Aromatic diamidine trypanocide	150
Doramectin	Avermectin anthelmintic agent	15
Eprinomectin	Avermectin anthelmintic agent	20
Febantel/Fenbendazole/Oxfendazole	Phenylguanidine/ Benzimidazole anthelmintic agent	100
Gentamicin	Aminoglycoside antibiotic	200
Imidocarb	Carbanalide antiprotozoal agent	50
Isometamidium	Trypanocide	100
Ivermectin	Avermectin anthelmintic agent	10
Lincomycin	Lincosamides antimicrobial	150
Monensin	Polyether ionophores antimicrobial	2
Neomycin	Aminoglycoside	1500
Pirlimycin	Lincosamide antibiotic	100
Spectinomycin	Aminocyclitol antibiotic	200
Spiramycin	Macrolide antimicrobial	200

Sulfadimidine	Sulfonamide antimicrobial	25
Thiabendazole	Benzimidazole anthelmintic agent	100
Tylosin	Macrolide antimicrobial	100
Procaine benzyl-penicillin	Penicillin antibiotic	4

Summary of Published Antibiotic Use Studies in India (Dairy)

Study	Population	Findings
Ramakrishna and Singh, 1985	203 raw milk samples from markets (152) and the National Dairy Research Institute (51) in Haryana	5.9 percent of the samples from the market and 3.9 % of the samples from the National Dairy Research Institute contained 10-20 µg/ml of streptomycin.
Sudershan and Bhat, 1995	205 milk samples from dairy farms in Hyderabad and Secunderbad and 12 surrounding suburban villages	• Interviews with 155 dairy farmers (38 urban and 117 rural) found that use of oxytetracyline was 55 percent and 20 percent in urban and rural farms respectively. • 205 milk samples (97 from individual buffalos, 101 from the market, and 7 from government organized dairies) were analysed for oxytetracycline residues. • 73 percent of animal samples,

		9 percent of market samples, and none of the government dairy samples contained these residues.
Unnikrishnan, Bhavadassan, Nath, and Ram, 2005 Study Date: 2000	Survey of farms in Bangalore and surrounding areas	• Tetracycline, gentamicin, ampicillin, amoxicillin, cloxacillin, and penicillin were commonly used for treatment of dairy animals. • Common treatment for mastitis was found to be beta-lactams or beta-lactams in combination with streptomycin.
G. Dutta, R. Dutta, Buragohain, and Mili, 2001	Five pooled milk samples from public milk booths in Guwahati, Assam	Two of the samples contained high levels of antibiotics (the equivalent of 5 µg/ml of penicillin), while 3 of the samples did not contain any antibiotics.
Ram, Bhavadasan, and Vijya, 2003	Milk from individual animals (125 cow and 87 buffalo), farms (93 organized and 89 unorganized), tankers (385), and pasteurized branded samples (650)	• Beta-lactam and tetracycline were found in 2.4 percent of the individual cow samples. None of the individual buffalo samples contained antibiotics. • Of the samples collected from farms, 5.4 percent of the organized samples and 2.2 percent of the unorganized samples contained beta-lactam and tetracycline residues. • 3.9 percent of the tanker milk supplies had beta-lactam

	were collected from southern India.	residues; tetracycline, streptomycin, and gentamicin were not detected. • 0.61 percent of the pasteurized milk samples contained beta-lactam antibiotic drugs, and no other antibiotic drugs were detected. • 3.9 percent of tanker milk samples received at six commercial dairies in southern India contained antibiotic residues.
National Dairy Research Institute, 2011 Data collected: 2010	44 raw milk samples from Delhi and surrounding villages.	• 11 percent contained beta-lactams, 2 percent contained streptomycin, and overall antibiotic incidence rate was 14 percent. • No gentamicin, tetracycline, or erythromycin detected.
Antibiotic Use and Resistance in Food Animals, Current Policy and Recommendations, Center for Disease Dynamics, Economics & Policy, 2016.		

NON RESTRICTIVE USE OF ANTIBIOTICS IN DAIRY COWS: CONCERNS

1. Public Health Aspects

Food Safety and Standards Act, 2006 defines veterinary drug residues as "the parent compounds or their metabolites or both in any edible portion of any animal product and include residues of associated impurities of the veterinary drugs concerned" (FSSA, 2006). The presence of residues may be the result of failure to monitor the withdrawal periods, illegal or off-label use of drugs and incorrect dosage levels or dosing schedule. Occurrences of veterinary drug residues pose the broad range of health consequences in the consumers. The residues of anti-bacterial may cause pharmacological, toxicological, microbiological and immune pathological health risks for humans.

a. Penicillins especially, as well as other ß-lactam antibiotics such as cephalosporins and carbapenems could cause allergies if high levels of residues persist in milk. Penicillin is not inactivated by pasteurization or drying and levels as low as 0.03 IU/ml has caused skin rashes.

b. Chloramphenicol causes disruptions like aplasia of the bone marrow.

c. Tetracyclines residues also have the potential to stain teeth of young children. Tetracyclines can react with nitrite to produce nitrosamines which is a carcinogen.

d. Development and spread of antibiotic resistance represents a serious threat with potential public health implications.

   Note: The isolation of bacterial pathogens of animal and human origin that are increasingly resistant to most frontline antibiotics, including third-generation cephalosporins, aminoglycosides, and even fluoroquinolones.

e. Pathological effects produced by Antibiotic Residues in Milk are as follows-

   i. Carcinogenicity by Sulphamethazine, Oxytetracycline, Furazolidone
   ii. Mutagenicity
   iii. Nephropathy (Gentamicin)
   iv. Hepatotoxicity
   v. Reproductive disorders

2. Technological aspects

Antibiotic residues in milk are undesirable from a manufacturing perspective, as they can interfere with starter culture activity and hence disrupt the manufacture process. The concentrations of antibiotics which would cause such effects is however often higher than would be found inherent as residues in milk.

a. Total inhibition of the starter culture has been observed to occur at approximately 60 µg/kg Penicillin G.

b. In the fermented dairy products such as cheeses and yogurts, the presence of antimicrobial agents can lead to the partial or total inhibition of the lactic bacterial growth.

c. Antibiotic residues can also interfere with the methylene blue test, intended to estimate the total microbial load in milk. The time taken for reduction of the dye will be increased, hence causing under estimation of the microbial load.

All of these concerns may result in major economic losses to the dairy industry.

TESTING OF RESIDUAL ANTIBIOTICS IN MILK AND MILK PRODUCTS

At both national and international levels, increasing attention is paid to the evaluation of the risk of occurrence of veterinary drug residues in foodstuffs and foods of animal origin, and to the introduction of appropriate measures to reduce this risk. The design and strategy of antibiotics and sulphonamide detection in milk involve two different aspects:

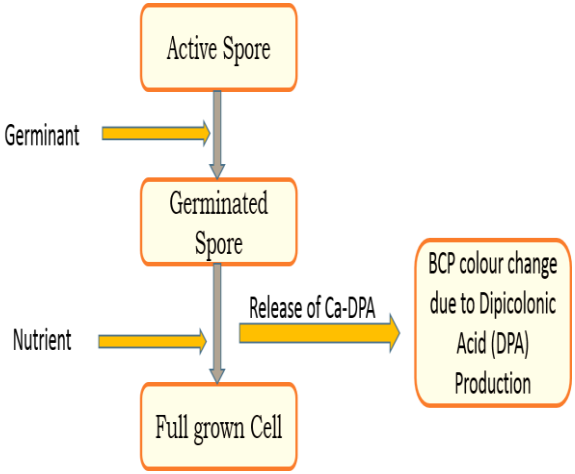
- the ability to sell the milk depending on its quality (technological safety), and
- the health safety of the milk regulated by the recent legislative regulations (toxicological safety).

1. DELVOTEST SP

DELVOTEST is the best known microbial inhibitor test. Its first version was developed, in the 1970s as Delvotest P, to detect β-lactams. The target organism, *B. stearothermophilus*, is encapsulated in an agar medium containing a pH indicator, a nutrient tablet and milk sample both being dispensed onto the agar surface. The 'ampoule version' is designed for individual tests or small-scale testing whilst a micro-tire plate version is designed for mass testing where 96 tests can be undertaken simultaneously. A negative result is indicated by a color change from purple to yellow, due to acid development during incubation at 64°C for 2½ hours.

The Delvotest P has been used throughout the world and has sensitivity to penicillin G of 0.005 IU/ml. A more recent development, the Delvotest SP, is capable of detecting a wider spectrum of substances, notably sulphonamides, but also has increased sensitivity to tylosin, erythromycin, neomycin, gentamicin, trimethoprim and other antimicrobials. The Delvotest SP appears identical to the Delvotest P, the only difference being the need to incubate the Delvotest SP for 2¾ hours. The Delvotest SP is sold throughout the world and, universally, has sensitivity to penicillin G of 0.003-0.004 IU/ml.

Antibiotic detection Based on inhibition



Test procedure

The growth of *B. stearothermophilus* spores at 64°C initiates an acidification process which causes the turning of a pH indicator from purple to yellow. The presence of antibacterial substances will cause delay or inhibition of the spores, depending on the concentration of the residues. In the presence of residues the spores will not multiply and the pH indicator will remain purple. Following steps are involved in procedure:

1. Add 1 nutrient tablet to each of the agar wells in the strip.

2. Inoculate 100 µl of milk into the agar well plus nutrient tablet. Seal the wells for incubation.
3. Incubate the strip of wells in a water bath at 64°C±0.5°C for 2.50 hours (at the time the negative control has been changed to yellow)
4. Examine the strip for colour change from purple to yellow. A yellow reading indicates that no inhibitory substances are present; a purple reading indicates that antibiotic residues are present and a yellow/purple reading indicates a doubtful result.

2. DELVOTEST® SP NT

Delvotest SP-NT, which is a non-specific microbial inhibitor test, In short this is an agar diffusion test that contains a standardized number of Bacillus *stearothermophilus* var. calidolactis spores, selected nutrients, and pH indicator bromocresol purple. After adding milk sample directly to the agar bed (ampoules), an incubation step was conducted for 3 h at 64 °C. During incubation, microbial metabolism resulted in a change in pH, and hence in a change of color from purple to yellow. By contrast, if the sample contained sufficiently high concentrations of inhibitory substances, the color would remain purple.

Procedure:

a. Preheat the incubation device-the temperature of the dry incubator or water bath should be set at 64 ± 2°C.

b. Select the required number of test materials-Detach one or more ampoules, or break the plate

in blocks depending on the number of milk samples to be analyse. Use scissors or a knife if needed. Take care that the aluminium foil from the remaining test is not damaged, if so, the test would be dry out. Return the remaining tests in appropriate storage conditions immediately. Remove the aluminium foil from the plate or perforate the ampoules carefully.

c. Add the milk Sample- Milk samples should be homogenised and representative of the milk to be tested. If milk from individual animal to tested do not use the first milk drops. Milk all four quarters from the cow to be analysed and gently mix the milk without creating foam. A one way pipette should never be used for more than one milk sample. Small droplets from other samples are enough to contaminate clean samples. Pipette 0.1ml of sample in the test. For each sample use a new and clean pipette. Clearly indicate each test.

d. Incubate the test in the incubator- when using test in plates, cover the plates using the included adhesive foil. Incubate the test plates or ampoules in the preheated incubator or water bath. Incubate the test for 3 hours or use control time.

e. Reading- the colour should be read from the 2/3 part of the ampoule or from underneath the test plate. If the test is (partially) yellow, the test is negative: the milk analysed does not contain antibiotics or the antibiotic concentration is

below the detection sensitivity of the test. The result is positive when the test is completely purple: the sample of milk contains antibiotics at or above the detection sensitivity of the test. Optional Reading software may be used.

f. To determine control time: as a negative control, pipette 0.1ml of milk, free from antibacterial substances (Provided by DSM), in to the first ampoule. Start reading the colour result of the negative control milk sample (first well) after an incubation time of 2hours 15 minutes. If the colour has not changed to yellow, the test ampoules should be returned to the incubating device until a yellow reading of the negative control sample is achieved (it is recommended to repeat the reading at intervals of 5 minutes). When the colour of negative control ampoule has turned yellow, all other ampoules can be read.

g. Sensitivity: at control time the sensitivity of the Delvotest SP NT is for Penicillin G at 2ppb and Sulfadiazine at 100ppb. When using a fix time of 3hours, the sensitivity is for penicillin G at 2ppb and for Sulfadiazine at 150ppb.

h. The table below summarizes the sensitivity of Delvotest SP NT as validated by the AOAC>

Antibiotics	**Sensitivity**	**Antibiotics**	**Sensitivity**
Amoxicillin	2.5ppb	Cephapirin	5.8ppb
Ampicillin	3.0ppb	Pencillin G	1.5ppb

Except fermented milk, 10 mL of each raw milk sample to be heated at 80 °C for 10 min to destroy natural inhibitors lysozyme and lactoferrin.

3. Charm Test

CHARM I and II tests are qualitative microbial receptor assays. The CHARM I test developed for β-lactams in milk was the first rapid test recognized by the AOAC (Association of Official Analytical Chemists) with a test time of 15 minutes. In 1984–1985, the CHARM I test was further developed to a test for antibiotics including, apart from β-lactams, tetracyclines, sulfonamides, aminoglycosides, chloramphenicol, novobiocin, and macrolides.

The extended version the CHARM I test has been referred to as CHARM II test. The Charm II (Charm Sciences Inc., Massachusetts, USA) is a commercial scintillation-based detection system for chemical families of drug residues utilizing class specific receptors or antibodies in an immunobinding assay format.

CHARM II test is based on the irreversible binding reaction between the functional groups of anti-bacterial and receptor sites on or within the cells of the added microorganisms. The Charm II uses ^{3}H and ^{14}C tagged drug tracers with broadly specific binding agents in a receptor assay format. The tracer molecules and any

analyte(s) present in the analytical sample compete for the binding sites. This competition for the receptor sites prevents the radiolabelled antibacterial from binding. Thus, the more radiolabelled compound binds, the less analyte is in the sample.

Following the binding interaction the reaction is stopped and unbound tracer is separated from the tracer–binder complex via a centrifugation step. Following the centrifugation step, the pellet (containing the tracer–binder complex) is analyzed in a scintillation counter for one minute to give a counting result expressed as counts per minute (cpm). The higher the count, the less drug contamination in the sample and conversely, the lower the count, the more drug contamination present in the sample. The result can be simplified to a present/absent result using a control point.

4. Beta-Star test

The test involves a specific β-lactam receptor linked to gold particles. It is a dipstick test that detects penicillins and cephalosporins. The milk sample (0.2 ml) is added to a vial containing the test reagents (receptor protein linked to gold particles), mixed and incubated at 47.5°C in the incubator for 3 minutes.

During incubation, the receptor will react with the free β-lactams contained in the sample. After 3 min of incubation, the dipstick is added and incubation is continued (2 min at 47.5°C). The mixture is transferred

to a strip of immuno-chromatography paper where it migrates towards the test field. With milk samples free of β-lactam residues, the receptor protein will be captured by a biomolecule immobilised at the test field of the chromatography paper.

Since the receptor protein is linked to gold particles, the captured protein-gold complex will appear as a pink-coloured band. With the sample where the receptor protein has interacted with free β-lactam molecules, the receptor protein will not be captured at the test field and no band will occur.

The colour intensity of the test band is visually compared with that of the reference band: if the colour intensity of the test band is weaker than that of the reference band, the sample is classified as positive.

Test Procedure

a. Mix milk sample or control 25 times in seven seconds with a 1 ft. movement.

b. Gently tap the vial on a hard surface in order to assure all solid material is in bottom of vial

c. Carefully remove the cap and rubber stopper from the vial

d. Pipette 200 µl milk sample into the vial.

    i. Attach a 200 µl pipette tip to the pipettor.

ii. Draw up sample, avoiding foam and bubbles.
iii. Deliver the sample into the vial by depressing the plunger.
iv. Replace the rubber stopper in the vial.

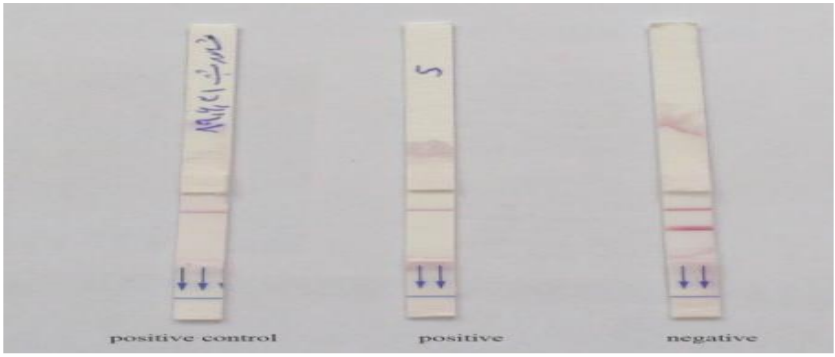
e. Mix the milk and reagent thoroughly by inverting the vial twice and swirl in a circular motion until all solids are in solution.

f. Remove stopper from vial and place the vial into the heater block and incubate at 47.5 ± 1.0°C for 3 minutes.

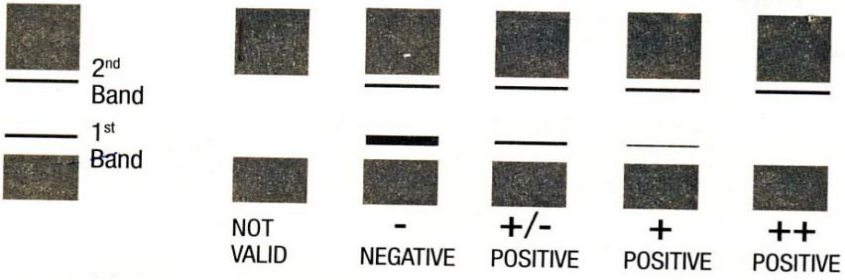
g. At the completion of the 3 minute incubation, place labeled dipstick into the vial in the heater block. The arrows on the dipstick must be oriented downward in the vial. Incubate the dipstick in the vial for 2 minutes at 47.5 ± 1.0°C.

h. At the completion of the 2 minute incubation, remove the dipstick from the vial, place the dipstick into the holder, and insert the holder into the reader. The dipstick must be read within 3 minutes.

Beta star Kit Interpretation



Strips Identification
Beta Star Kit Interpretation



Commercially available Milk Screening Tests

Test Name/Kit Name	Residues Detected At or Below Safe/Tolerance Levels
Charm Cow side II Test	Amoxicillin, Ampicillin, Cephapirin, Chlortetracycline, Hetacillin, Neomycin, Oxytetracycline, Penicillin, Pirlimycin, Tetracycline, Tilmicosin, Tylosin

Charm MRL Beta-lactam Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Hetacillin, Penicillin
Charm MRL Beta-lactam 3 Minute Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Hetacillin, Penicillin
Charm MRL Beta-lactam 1 Minute Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Hetacillin, Penicillin
Charm MRL Beta-lactam and Tetracycline Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Chlortetracycline, Hetacillin, Oxytetracycline, Penicillin, Tetracycline
Charm MRL Beta-lactam and Tetracycline 2 Minute Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Chlortetracycline, Hetacillin, Oxytetracycline, Penicillin, Tetracycline
Charm MRL Beta-lactam and RF Tetracycline 2 Minute Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Chlortetracycline, Hetacillin, Oxytetracycline, Penicillin, Tetracycline
Charm MRL Trio Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Chlortetracycline, Hetacillin, Oxytetracycline, Penicillin, Sulfachlorpyridazine, Sulfadiazine, Sulfadimethoxine, Sulfamerazine, Sulfamethazine, Sulfamethizole, Sulfaquinoxaline, Sulfathiazole, Tetracycline
Charm Quad Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Chlortetracycline, Dihydrostreptomycin, Hetacillin, Oxytetracycline, Penicillin, Streptomcyin, Tetracycline
Charm Quad1 Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Chlortetracycline, Hetacillin, Oxytetracycline, Penicillin, Tetracycline

Charm Quad2 Test	Erythromycin, Lincomycin, Pirlimycin, Tilmicosin, Tylosin
Charm Quad3 Test	Dihydrostreptomycin, Neomycin
Charm Blue Yellow II Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Chlortetracycline, Hetacillin, Lincomycin, Neomycin, Oxytetracycline, Penicillin, Pirlimycin, Tetracycline, Tilmycosin, Tylosin
BetaStar Plus Beta-lactam Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Cloxacillin, Penicillin
Charm II Beta-lactam Test (Competitive)	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Penicillin
Charm II Beta-lactam Test (Quantitative)	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Cloxacillin, Penicillin
Charm II Beta-lactam Test (Sequential)	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Penicillin
Charm B. stearothermophilus Tablet Disc Assay	Amoxicillin, Ampicillin, Cephapirin, Penicillin
Charm SL Beta-lactam Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Penicillin
Charm 3 SL3 Beta-lactam Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Cloxacillin, Penicillin
Charm Flunixin and Beta-lactam Test	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Cloxacillin, Flunixin, Penicillin
Charm II Test for Cloxacillin in Milk	Cloxacillin
Charm II Sulfa Drug Test (Competitive Assay)	Sulfadiazine, Sulfadimethoxine, Sulfamethazine, Sulfathiazole
Charm II Tetracycline Test	Chlortetracycline, Oxytetracycline, Tetracycline
Delvotest P 5 Pack	Amoxicillin, Ampicillin, Cephapirin, Penicillin

Delvotest P/Delvotest P Mini	Amoxicillin, Ampicillin, Cephapirin, Penicillin
Delvotest SP/Delvotest SP Mini	Amoxicillin, Ampicillin, Cephapirin, Penicillin
New SNAP Beta-Lactam Test Kit	Amoxicillin, Ampicillin, Ceftiofur, Cephapirin, Penicillin

Prevention of Antimicrobials in milk

The dairy industry is committed to producing safe, abundant, and affordable milk of the highest quality. When dairy animals get sick and treatment is necessary, producers and veterinarians use drugs judiciously. Antibiotics should be used appropriately to prevent residues from occurring in milk. Safe levels of residues in milk and other animal products result from the participation of all activities involved in the food chain from “stable to table”.

Possible strategies for prevention of antimicrobials residues in milk is as follows:-

1. Establish a valid veterinary/client/patient relationship (VCPR) to ensure proper diagnosis and treatment of disease.

2. Use of drugs approved for specific disease indications according to labelled recommendations. Establishment of pharmacokinetics and withholding time for antibacterial used in dairy animal to describe metabolism and distribution of drugs in

different tissue and milk. Withholding period to be valued by considering pharmacokinetics of a drug, formulation of drug, Combination of two drugs with same antibiotic.

3. Implement a preventive animal health program to reduce the incidence of disease.

4. Good hygiene and good management practices at farm and the milk processing units may lead to reduce the disease spreading among the livestock and this reduces the antimicrobials usage in the farms (Specially Mastitis management programme etc.,)

5. Evaluation and use of alternative to antibiotic growth promoter e.g. probiotic microorganisms, immune modulators, organic acids (acidifiers) and other feed supplements.

6. Establishing the use policy for antibacterial in animals will help for monitoring and surveillance of the usage of these drugs.

7. Pharmacovigilance programmes would be developed for veterinary pharmaceuticals concerning the safety of veterinary medicines used for the treatment, prevention or diagnosis of disease in animals.

8. Establishment of pharmacovigilance working group and an effective reporting system involving

veterinarians, immunologists, pharmacologists, toxicologists and eco-toxicologists is an important prerequisite for the risk assessment of antibacterial drug residues for human and environment.

9. Maintaining treatment records of cows in order to determine appropriate withholding periods. This will also help to treat dry cow with long acting substances so the withholding period can be adjusted if the dry period is shorter than expected.

10. Recommendations of the drug manufacturer regarding dosage, route of administration, treatment intervals and storage condition of antimicrobials should be followed intimately because any deviation may contribute to extended withholding periods.

11. Development and validation of rapid screening tests for detection of antimicrobial residues in milk at individual cow basis to make sure that milk of individual cows is free of inhibitors after the end of the withholding period.

There is still a lack of regulations and guidelines regarding use of antibiotics in veterinary practice in India. The issue of antibiotic residues in food chain warrants the further policies and guidelines to address the possible risk to public health and environment.

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